Prediction studies of environment-friendly biodegradable polymeric packaging based on PLA. Influence of specimens' thickness on the hydrolytic degradation profile

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Abstract

Application of new biodegradable polymer packaging based on polylactide (PLA), susceptible to organic recycling, can help in the waste reduction in landfills. In this paper, the results of the study on abiotic degradation of PLA and its blend containing 15 mol% of poly[(*R*,*S*)-3-hydroxybutyrate], as a model for the first step of organic recycling were presented. The samples used for this study have different shapes and thicknesses: rigid films and cuboid-bars. Particular emphasis was placed on determining the pattern of degradation products released into the medium. Originally, the results of present study revealed that the application of electrospray ionization mass spectrometry supported by high performance liquid

chromatography allowed envisaging the differences in the degradation products pattern released from the studied PLA-based samples differing in thickness. The significant differences in degradation products pattern were predominately observed in the first steps of incubation process and are caused by an autocatalytic effect, which occurs mainly during degradation of the large size PLA samples. Although, the thickness of PLA-based packaging changes the degradation product patterns, however this does not increase the total amounts of acids released to the medium. Thus, it may be concluded that thickness should not affect significantly organic recycling of the packaging.

Keywords: polylactide, biodegradable polyesters, hydrolytic degradation, liquid chromatography, ESI-MS, HPLC-DAD

1. Introduction

Bioplastics still only account for 1 % of global plastics production. Thus, we are just at the beginning of cleaning up what has been done by the development of classical plastics. However, there is an awareness that plastic waste will continue to grow with increased population. [Jambeck et al., 2015] It is therefore a growing interest in new materials with good processing properties that can be used as a packaging and which help to preserve the current state of natural environment [Grizzi et al., 1995; Musioł et al., 2016a, 2016b; Iovino et al., 2008; Sikorska et al., 2008; Davis, 2003]. An interesting alternative for packaging from conventional plastics could be the polymers susceptible to degradation by biological activity. Biodegradable polymers includes natural polymers from plant like starch, polymers produced by microorganisms (polyhydroxyalkanoate, PHA), polymers whose monomers have natural origin (polylactide, PLA) and mainly synthetic polymers such as aliphatic-aromatic copolyesters [Rychter et al., 2006, 2010, 2011, 2016; Musioł et al., 2011; Rydz et al., 2015a;

Song et al., 2009]. It is expected that in the near future, interest in polyester materials based on renewable raw materials and their organic recycling will increase in the packaging and agricultural sectors. [Rydz et al., 2015b; Żenkiewicz et al., 2013; Sikorska et al., 2015; Castro-Aquirre et al., 2017; Sikorska et al., 2012]

To evaluate the influence of the formation method on the degradation profile of biodegradable polymers, the hydrolytic degradation of the nonwoven materials made from poly(lactic-*co*-glycolic acid) (PLGA) has been studied in water at 70 °C. The analysis of the water-soluble degradation products was performed by high performance liquid chromatography (HPLC) and electrospray mass spectrometry (ESI-MS) techniques. [Sikorska et al., 2014]

The preliminary HPLC results and the available literature data suggest the possibility of the use of HPLC technique to determine the amount of acids and oligomers release from the materials studied [Sikorska et al., 2014; Li et al., 2007; Watanabe et al., 2012]. This technique has also been successfully used to analyze the lactide, glycolide, lactic and glycolic acids content in polymers and to measure the purity of the intermediate lactide in the production of PLA [Codari et al., 2011; Zamanova et al., 2014; Feng et al., 2009]. Bachmann and Seebach published the HPLC-based method for separation and detection of 3-hydroxybutyric acid (3-HBA) oligomers after derivatization with diazomethane to the corresponding methyl esters [Bachmann and Seebach, 1999]. The procedure for fatty acids derivatization with bromophenacyl bromide which allows separation of 3-HBA and its oligomers without cleavage of the polymer chain and subsequent sensitive detection of the bromophenacyl-modified products was also elaborated [Gebauer and Jendrossek, 2006]. Li *et al*. and Feng *et al*. in theirs articles about the enzymatic degradation of poly(3-hydroxybutyrate) (PHB), poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) and poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) ($PHBHH_x$) showed that, in accordance with previous studies the use of HPLC to determine the water-soluble degradation products of these polymers are possible [Li

et al., 2007; Feng et al., 2004]. Thus, the HPLC technique could be used for practical determination of the degradation products of PHA or PLA and polyglycolide as well as their copolymers. Coupled methods of liquid chromatography were developed to discriminate macromolecules of distinct compositions or architecture independent of their molar mass; they combine entropic contribution as well as enthalpic interaction to retention of macromolecules. The well-known coupled methods are liquid chromatography under critical condition (LCCC) [Berek, 2016; Malik et al., 2012] and eluent gradient liquid chromatography (EGLC) [Murgasowa and Hercules, 2002; Beaudoin et al., 2008; Berek, 2000] as well as liquid chromatography under limiting condition of desorption (LCLCD) [Šišková et al., 2012, 2103; Rollet et al., 2014; Šišková and Berek, 2010].

Herein, the results of the study on abiotic degradation of samples with different thickness fabricated from PLA-based material are presented. The studies of degradation process were performed for the samples of PLA and its blend containing 15 mol% of poly[(*R*,*S*)-3-hydroxybutyrate], (*R*,*S*)-PHB, processed in two forms: rigid films with a thickness of 0.3 mm and cuboid-bars having a thickness of 5 mm. During the degradation process the micro- and macroscopic observations of the surface and changes in molar mass and composition of the samples were monitored.

The main research problem undertaken in this work was to determine the effect of specimen's thickness on the degradation products patterns released from them. The structural characterization of the water-soluble degradation products of the samples studied were determined with the aid of multistage mass spectrometry (ESI-MS^n) analysis. Additionally, the amounts of acids released from the samples studied after a specific period of degradation was measured with the aid of high performance liquid chromatography with UV/diode array detector (HPLC-DAD) by use of the calibration curve based on both lactic acid and 3-hydroxybutyric acid standards.

2. Materials and methods

2.1. Materials

Commercial polylactide type 2002D (NatureWorks®, USA) with melt flow rate (MFR) of 5-7 g/10 min (2.16 kg, 210 °C), density d = 1.24 g(cm)$^{-3}$ and content of *D*-mers below 3.5 %. For forming the materials studied was used the pellets with average molar mass (M_w) and dispersity ($Đ_M$) equal 168 000 g(mol)$^{-1}$ and 1.3, respectively. [Kolstad et al., 2012] The high-molar mass poly[(*R*,*S*)-3-hydroxybutyrate] with M_w = 100 000 g/mol was obtained in laboratory scale *via* anionic ring-opening polymerization (ROP) of racemic *ß*-butyrolactone in the presence of tetrabutylammonium acetate as an initiator, using original method elaborated in Centre of Polymer and Carbon Materials PASci. The ROP was carried out in bulk at room temperature. The resulting polymer has been dissolved in chloroform and protonated. Then after precipitation in hexane (*R*,*S*)-PHB was dried under vacuum at 40 °C. [Kurcok et al., 2002]

Degradation process was performed for the samples of PLA and its blend containing 15 mol% of (*R*,*S*)-PHB. The samples were prepared in the form of rigid films with average thickness of 0.3 mm (denoted as PLA/F and PLA/(*R*,*S*)-PHB/F) and cuboid-bar samples having average thickness of 5 mm (denoted as PLA/C and PLA/(*R*,*S*)-PHB/C). The melt-mixing of blend component was performed in extruder. For the film preparation the melt components were additionally casting on chill rolls. [Bartczak et al., 2013]

2.2 Abiotic degradation under the laboratory conditions

For the degradation experiments samples with the dimension of 30 x 10 mm were dried under vacuum at room temperature to a constant mass to eliminate ultimate water content and were then incubated at 70 °C (±0.5 °C) in screw-capped vials with air-tight PTFE/silicone septum, containing 25 cm^3 of distilled water. The degradation experiment was conducted over 180 days as was previously described [Musioł et al., 2011].

2.3 Imaging of sample texture

Macroscopic changes of the surface of the tested materials were visualized using a digital camera Olympus E-410, while microscopic changes were analyzed using an optical microscope. Analyses were performed using a Zeiss polarizing microscope (Opton-Axioplan) equipped with a digital camera Nikon Coolpix 4500 color. Photomicrographs were taken at a magnification of 120x.

SEM studies were performed using a Quanta 250 FEG (FEI Company, USA) high resolution environmental scanning electron microscope operating at 10 kV acceleration voltages. The samples were observed without coating under low vacuum (80 Pa) using a secondary electron detector (Large Field detector).

2.4. Gel Permeation Chromatography (GPC) analysis

The GPC experiments for the samples studied were conducted in chloroform solution at 35 ºC and a flow rate of 1 mL(min)$^{-1}$ using a Spectra-Physics 8800 solvent delivery system with two Mixed C PL-gel styragel columns in series and a Shodex SE 61 refractive index detector. A volume of 10 µL of sample solutions in $CHCl_3$ (concentration 0.5 % w/v) were injected into the system. Polystyrene standards with low dispersity were used to generate a calibration curve.

2.5. High-performance liquid chromatography coupled with UV/diode array detector (HPLC-DAD)

The water-soluble degradation product analyses were performed with high-performance liquid chromatography using a Smartline System (Knauer, Berlin, Germany) HPLC equipped with a Knauer Smartline 1000 pump, Knauer A 1357 injection valve and a Knauer UV 2600 multi-wavelength diode array detector set at 210 nm (UV/DAD).

The sample was introduced through a 20 µL loop on a Hamilton PRP-X300 analytical column (7 µm, 150 x 4.1 mm i.d.) with an analytical guard column (2.3 x 20 mm, Hamilton) and a

151 water mobile phase containing 0.01 $molL^{-1}$ sulfuric acid (HPLC grade). Separation
152 conditions: isocratic, flow rate 0.5 $mL(min)^{-1}$ and ambient temperature. The calibration curve
153 was calculated based on the lactic acid and 3-hydroxybutyric acid standards (Figure 1).

154 <Figure 1>

155 **2.6. Proton nuclear magnetic resonance spectroscopy (^{1}H NMR)**

156 The ^{1}H NMR spectra were recorded using a Varian VXR-300 multinuclear spectrometer. The
157 spectra were obtained in $CDCl_3$ using tetramethylsilane (TMS) as an internal standard at 28
158 °C with 64 scans, 7 μs pulse width and 3.74 s acquisition time. The composition of starting
159 PLA/(*R*,*S*)-PHB blend samples as well as sample of PLA/(*R*,*S*)-PHB blends remaining after
160 specific period of abiotic degradation was determined based on integration of protons of
161 methyl groups originated from (*R*,*S*)-PHB and its degradation products trapped in the polymer
162 matrix: 3-hydroxybutyric acid, oligomers (OHB) and protons of methyl groups originated
163 from PLA and its degradation products trapped in polymer matrix: lactic acid (LA) [Musioł et
164 al., 2016].

165 **3. Results and Discussion**

166 Polyesters produced from renewable resources offer ecological advantages as compared to
167 commercially available plastics produced from fossil carbon sources. Selective use of
168 biodegradable packaging materials in certain applications may provide a solution of the
169 environmental problems. However, due to the wide spectrum of potential applications, some
170 limitations and difficulties concerned biodegradable polymers have appeared. Therefore, the
171 *ex-ante* and *ex-post* study are needed in the area of advanced biodegradable polymeric
172 materials in order to identify and minimize potential failures of new packaging products.
173 [Rydz et al., 2015b] The studies of abiotic degradation of PLA and its blend containing 15
174 mol% of poly[(*R*,*S*)-3-hydroxybutyrate], as a model of the first step of organic recycling, was
175 performed. To determine difference in degradation products patterns the two forms of

materials were used: rigid films (PLA/F and PLA/(*R*,*S*)-PHB/F) with a thickness of 0.3 mm and cuboid-bar samples (PLA/C and PLA/(*R*,*S*)-PHB/C) having a thickness of 5 mm.

Figure 2 presents the digital photographs of PLA and PLA/(*R*,*S*)-PHB blend samples before and after specific period of incubation in water at 70 ºC. The samples surface before degradation, despite their form, was smooth without indentations and pits. However, the blend samples PLA/(*R*,*S*)-PHB/F and PLA/(*R*,*S*)-PHB/C show greater turbidity opposite to PLA samples. After 14 days of incubation the surface of blend cuboid-bar sample PLA/(*R*,*S*)-PHB/C began to crumble and the layer structure was observed. Moreover, delamination of specimens until 56^{th} day of incubation was noticed. In contrary, the surface of PLA/C cuboid-bar sample becomes rougher from the beginning of the incubation process with appearing of pores and pinholes like in case the film samples PLA/F and PLA/(*R*,*S*)-PHB/F. During whole period of incubation time the cracking process and gradually mass loss of the samples was also observed (see Figure 2, Tables 3 and 4). After longer incubation period the disintegration and in the consequence disappearance of all samples was noticed. The tarnishing observed for PLA/F and PLA/C samples confirm their crystallization [Sikorska et al., 2012].

<Figure 2>

The blend samples surface changes observed in the macroscale were confirmed on the nanometer scale by SEM analysis. Figure 3 shows the SEM images of samples studied before and after specific period of incubation in water at 70 ºC. The assessment of the results obtained from SEM revealed changes in surface texture. After degradation process on the surface the damages were appeared while the starting samples were smooth.

<Figure 3>

Moreover, from the images of fracture areas can be seen that, the internal part of PLA/(*R*,*S*)-PHB/C cuboid-bar samples forming lamellar structure (Figure 4).

<Figure 4>

The cracking process goes layer by layer and induce the formation of specific structure which facilitating the penetration of water inside the samples. The swelling process is also responsible for the trapping of degradation products inside cuboid-bar samples (Figure 4). In the case of PLA/(*R*,*S*)-PHB/F film sample the tendency to the delamination and the microcracks formation was noticed after 14 days of incubation in water. The delamination means that microcracks in external part of PLA/(*R*,*S*)-PHB/F film sample was bigger than ones in internal part. The internal part of sample shows smaller changes probably due to the fact that water does not goes into the material as in case of PLA/(*R*,*S*)-PHB/C cuboid-bar sample and cracking process progressing faster in the external part of the PLA/(*R*,*S*)-PHB/F film sample, however, layer by layer (Figure 5).

<Figure 5>

After longer incubation period the appearance of pinholes and pits on the PLA/(*R*,*S*)-PHB/F film surface was observed (see Figure 6).

<Figure 6>

To determine the changes in the chemical composition of the samples studied during the incubation in water the NMR analysis was applied. The ^{1}H NMR analysis of solid part of PLA/(*R*,*S*)-PHB/F and PLA/(*R*,*S*)-PHB/C samples (remaining after specific period of abiotic degradation) was performed and the results were collected in Table 1.

<Table 1>

Results in Table 1 show differences in composition of samples after specific period of degradation, depending on their thickness. After 14 days of incubation the highest changes in composition was observed for PLA/(*R*,*S*)-PHB/C cuboid-bar sample. The faster loss of the PLA component in the case of PLA/(*R*,*S*)-PHB/C cuboid-bar sample could results from significantly acceleration of the rate of hydrolysis of PLA component by the autocatalytic effect occur predominantly in thicker samples [Grizzi et al., 1995; Espartero et al., 1996].

226 The changes of number-average molar mass and dispersity for the investigated samples
227 during degradation process in water at 70 ºC have been monitored using the GPC analysis.
228 The obtained results are presented in Table 2 and in Figure 7.

229 <Table 2>

230 <Figure 7>

231 The GPC analyses indicated the shift to lower molar mass values for all samples studied after
232 specified time of incubation. However, in the case of cuboid-bar samples the effect was
233 slightly larger. It may indicate an autocatalytic effect which can occurs during hydrolytic
234 degradation process of thicker materials [Espartero et al., 1996]. Vert group originally
235 reported that the hydrolytic degradation of aliphatic polyesters derived from lactic acid
236 proceeds heterogeneously in the case of large size devices [Grizzi et al., 1995]. Thus, the rate
237 of degradation being greater inside than at the surface, which is reflected by the GPC traces.
238 For the PLA samples the autocatalytic effect is observed only at the beginning of degradation
239 process of PLA/C cuboid-bar sample. After longer incubation time the molar mass was
240 reached the same value for both kinds of samples: rigid film (PLA/F) and cuboid-bar (PLA/C)
241 (see Table 2). With progress of hydrolytic degradation of PLA/(*R*,*S*)-PHB/F and PLA/(*R*,*S*)-
242 PHB/C samples the water soluble degradation products could be released into the degradation
243 medium where their structure can change further with the progress of incubation time. The
244 structure of the water-soluble products formed during the hydrolytic degradation of the
245 PLA/(*R*,*S*)-PHB/F and PLA/(*R*,*S*)-PHB/C samples which were released to the water, was
246 determined using ESI-MS and HPLC-DAD techniques. [Sikorska et al., 2014; Adamus, 2007;
247 Rydz et al., 2013] Figure 8 shows ESI-mass spectra (positive mode) of water medium
248 collected after 14 and 56 days of incubation of PLA/(*R*,*S*)-PHB/F rigid film and PLA/(*R*,*S*)-
249 PHB/C cuboid-bar samples.

250 <Figure 8>

Regardless of the form of tested samples PLA/(*R,S)*-PHB/C or PLA/*(R,S)*-PHB/F two series of singly charged ions that regularly occurred at every 72 Da (monomeric unit in PLA, Series A) and 86 Da (monomeric unit in (*R,S)*-PHB, Series B) were observed in the mass spectra of degradation mediums obtained after 14 and 56 days of incubation of samples studied (see Figure 8). These two series of ions represent the degradation products of two blend components with different degree of oligomerization. [Musioł et al., 2016] The series of ions occurring at $m/z = 89+(72\times n)+23+1$, labeled as Series A (Figure 8) correspond to sodium-cationized lactic acid oligomers terminated with hydroxyl and carboxyl end groups. The second series of ions occurring at $m/z = 103+(86\times n)+23+1$, labeled as Series B (Figure 8) can be assigned to sodium-cationized oligo(3-hydroxybutyrate) with hydroxyl and carboxyl end groups. The chemical structures of the ions (Series A and B) appearing in the mass spectra are depicted in Figure 8. The structural assignment of the molecular ions (Series A and B) which represent the degradation products of two blend components was confirmed by the ESI-MS/MS experiment. The fragmentation experiments were performed for the selected molecular ions at m/z = 557 and at m/z = 545, respectively for OHB and OLA (Figures 9 and 10).

<Figure 9>

<Figure 10>

The fragmentation experiment confirms that the main products that can migrate from blends to the water medium are the oligomers: OLA and OHB terminated by hydroxyl and carboxyl end-groups. [Sikorska et al., 2012; Hakkarainen and Albertsson, 2008] However, it is worth noting that the comparison of the mass spectra obtained for the mediums in which the PLA/*(R,S)*-PHB/C and PLA/*(R,S)*-PHB/F samples were degraded indicate on the effect of specimen thickness on degradation products pattern released from them (see Figure 8). The visible differences in intensities of the series A on mass spectra obtained were observed

especially at the first incubation period (14 days). The intensity of the signals of series A corresponding to the lactic acid oligomers are lower in the case of medium were the PLA/*(R,S)*-PHB/C cuboid-bar sample was incubated. Lower intensity of the PLA oligomers observed in this case is probably caused by autocatalytic effect observed during degradation of thicker PLA samples. Water that penetrates deep into the sample initiates the degradation process of the PLA component. The lactic acid formed acts as an autocatalyst which accelerate the hydrolysis of PLA component deeply inside the sample and the degradation products formed (OLA oligomers and mainly the lactic acid). In the first stage, the resulting degradation products are blocked in the sample matrix and their migration to the degradation medium is hindered. As the degradation process in the outer layers of the sample progresses, the products (mainly lactic acid) are successively released into the medium. The effect is not observed for the thin film PLA samples. This observation is consistent with the SEM results (see Figures 4 and 5). Additionally, during the incubation time of PLA/*(R,S)*-PHB/C and PLA/*(R,S)*-PHB/F samples the signals both of OLA and OHB moved to lower value of m/z what suggest progress of degradation process of both blend components, wherein the PLA component of the blends degraded faster compared to the PHB component (see results after 56 days of incubation in Figure 8). From the ESI-MS spectra presented on Figure 8 it is difficult to estimate the amount of the released of lactic and 3-hydroxybutyric acids. It is known that when applying ESI-MS to mixtures containing both acids and oligomers, the intensities of the signals corresponding to acids are lower than those of the oligomers. The quantitative estimation of the oligomer and acid contents would require separate calibrations for each component type. Therefore, the amounts of the released lactic and 3-hydroxybutyric acids were separately determined using HPLC and results were presented in Figure 11 as well as in Tables 3 and 4.

<Figure 11>

<Table 3>

<Table 4>

The HPLC analysis of water soluble degradation products of PLA samples, regardless their thickness, shown that the amount of lactic acid in the medium increased during progress of degradation process from 0.08 $molL^{-1}$ after 14 days to 0.88 $molL^{-1}$ after 180 days (Table 3). Growing concentration of released lactic acid from both types of samples tested corresponds with mass loss of PLA samples during degradation. The HPLC analysis of water soluble degradation products of PLA/*(R,S)*-PHB/F and PLA/*(R,S)*-PHB/C samples indicate the faster initial release of lactic acid from the cuboid-bar sample PLA/*(R,S)*-PHB/C. After 14 days of degradation the concentration of lactic acid in degradation medium was four time higher compare to the sample of PLA/*(R,S)*-PHB/F film (Table 4). The presence of higher amount of lactic acid (Figure 11) and smaller amount of lactic acid oligomers (Figure 8) observed in water soluble degradation products of PLA/*(R,S)*-PHB/C cuboid-bar sample confirmed the autocatalytic effect which can occurs during first step of hydrolytic degradation process of thicker PLA-based sample. The initial measurable amount of 3-hydroxybutyric acid has been noticed after 56 and 28 days of degradation of PLA/(*R,S*)-PHB/F and PLA/(*R,S*)-PHB/C samples, respectively. The normalized acid concentration was almost two times higher for thicker PLA/(*R,S*)-PHB/C cuboid-bar sample after 56 and 70 days of degradation when compared with blends in form of thin film. After longer degradation time the growing amount of both acids was observed. Increasing concentration of degradation products of both blends components corresponds to the mass loss of samples during incubation time. Higher concentration of leached lactic acid as well as faster initial 3-hydroxybutyric acid release from thicker cuboid-bar sample PLA/(*R,S*)-PHB/C into medium results probably also from the processing method of the sample. The surface imperfections observed in case of PLA/*(R,S)*-PHB/C cuboid-bar sample (see Figures 3 and 4) facilitate the water permeating inside the

samples which allow to formation of local “micro-reactors” where hydrolyze of PLA component leads to lactic acid. The lactic acid formed acts as an autocatalyst which accelerate the PLA hydrolysis mostly to the lactic acid. The small effect of processing on mechanical and thermal properties of PLA specimens as well their degradation was observed [Sikorska et al., 2012; Żenkiewicz et al., 2009]. Furthermore, this observation is in accordance with the results of SEM analysis (see Figures 3-6).

Conclusion

The results of the presented study revealed that the thickness and method of production of polymeric packaging based on PLA has a significant impact on their degradation rate as well as on the pattern of degradation products released from them. The application of ESI-mass spectrometry supported by HPLC analyses for the monitoring of hydrolytic degradation of PLA/(*R*,*S*)-PHB/F and PLA/(*R*,*S*)-PHB/C samples allowed to confirmation of the release of water-soluble products into the degradation medium and establish their chemical structure at molecular level. As a water-soluble degradation products were identified the lactic and 3-hyroxybutyric acids as well as their oligomers, OLA and OHB terminated by hydroxyl and carboxyl end-groups. The analytical scheme used in the study allowed to visual envisage the differences in the pattern of degradation products released from the samples studied dependent on their thickness. The significant differences in degradation products pattern were predominately observed in the first steps of incubation process. The lower intensity of the signals corresponding to PLA oligomers observed in the case of the degradation of PLA/(*R*,*S*)-PHB/C cuboid-bar sample was caused by the autocatalytic effect, occurring during degradation of the thicker PLA samples. However, this effect does not result in the increase of the total amounts of acids released to the medium. It means that the thickness of PLA-based materials changes their degradation profile but does not impact on environment by increasing the acidic products being a resulting from organic recycling of packaging fabricated from

PLA. Thus, the results of the presented study can be useful for the design of new environment-friendly biodegradable PLA-based packaging.

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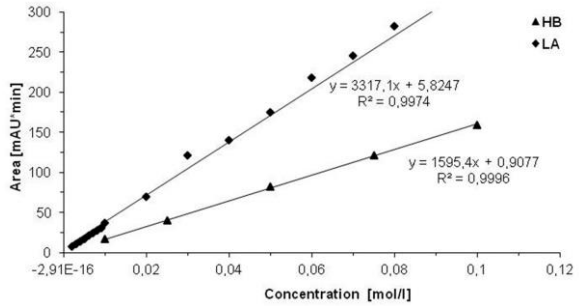
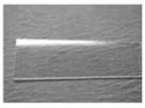
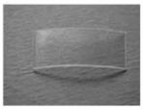
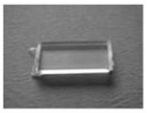
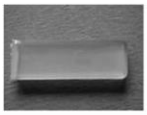
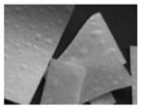


509

510 *Figure 1. Calibration curve for lactic acid (LA) and 3-hydroxybutyric acid (HB) standards*

511

512

Incubation time [days]	Sample name			
	PLA/F	PLA/(R,S)-PHB/F	PLA/C	PLA/(R,S)-PHB/C
0				
14				
56				

513

514 *Figure 2. The digital photographs of samples studied before (0) and after specified times of*

515 *incubation (14 and 56 days)*

516

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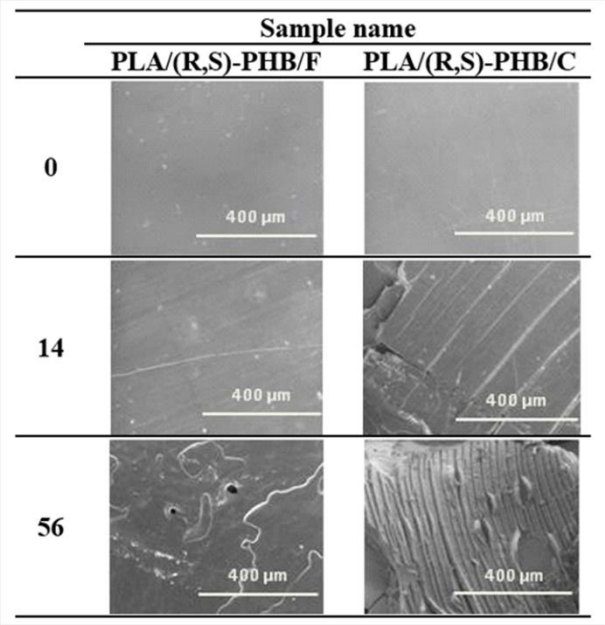


518

519 *Figure 3. SEM images of blend samples surface before (0) and after specified times of*

520 *incubation (14 and 56 days)*

521

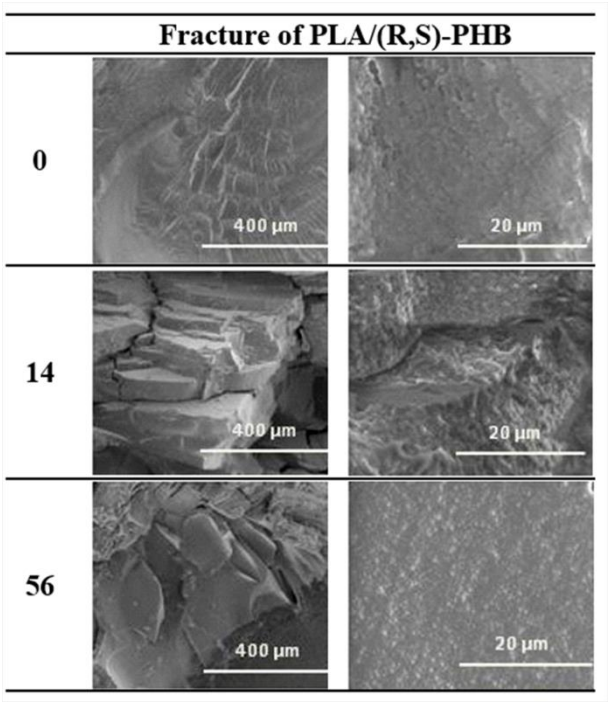
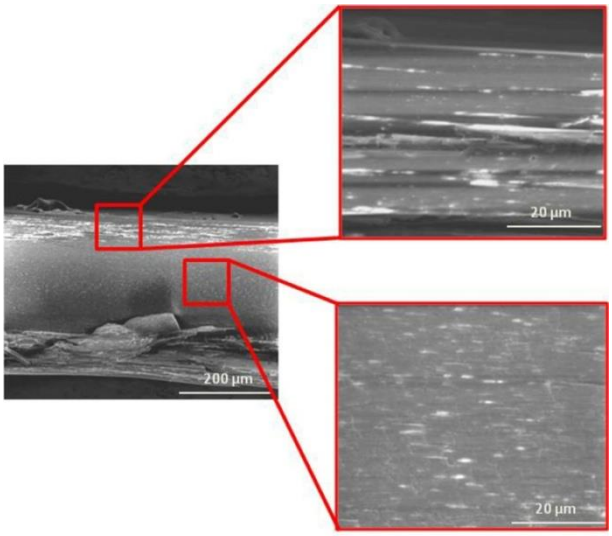


Figure 4. Fracture areas observed for PLA/(R,S)-PHB/C cuboid-bar samples studied before (0) and after specified times of incubation (14 and 56 days)

527



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529 *Figure 5. Fracture areas observed for PLA/(R,S)-PHB/F film after 14 days of incubation*

530

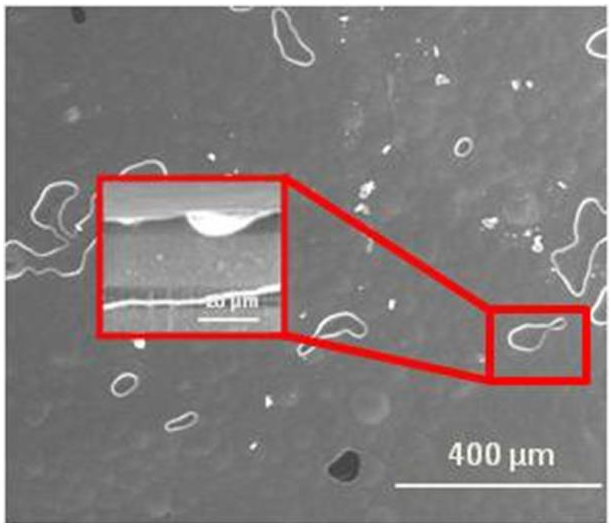

Figure 6. SEM image of surface of PLA/(R,S)-PHB/F film sample after 56 days of incubation with SEM image of fracture of selected surface area

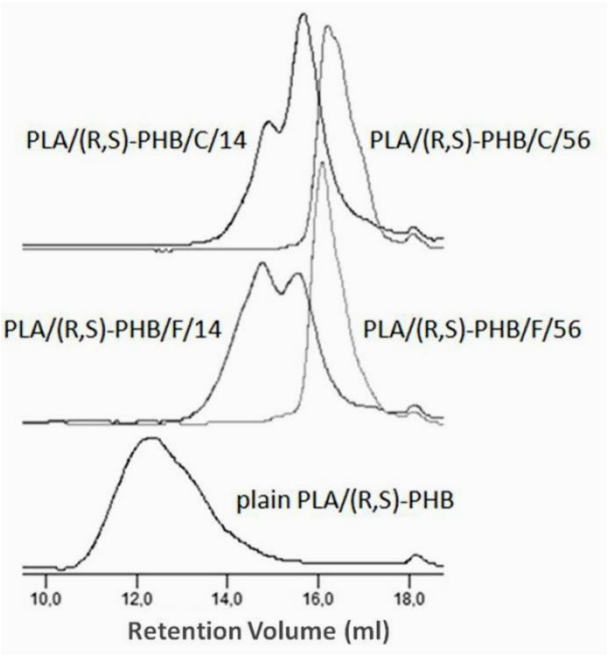

Figure 7. The GPC elugrams of the PLA/(R,S)-PHB samples before (plain) and after specified times of incubation (14 and 56 days)

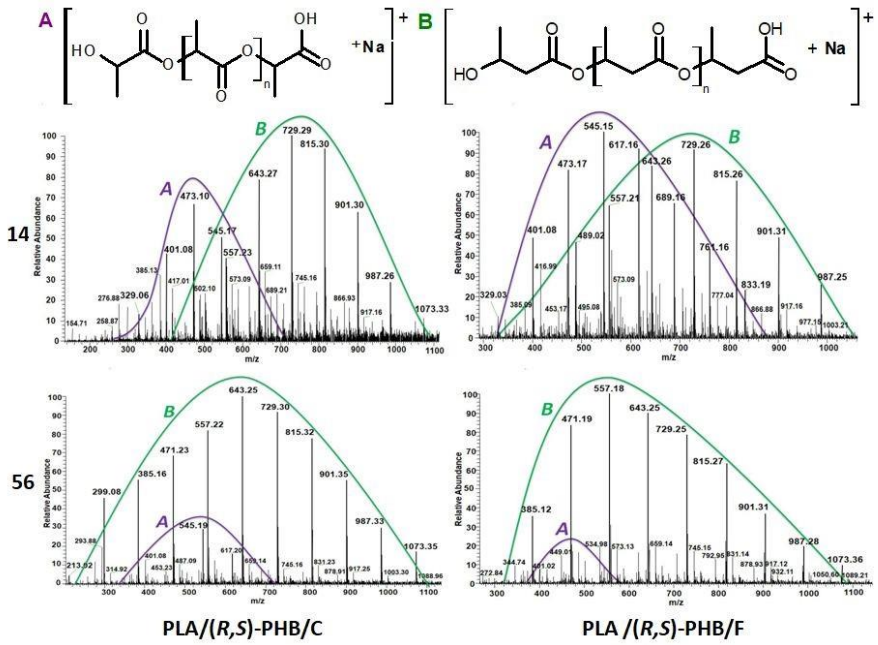

Figure 8. ESI-mass spectra (in positive-ion mode) of degradation products of the PLA/(R,S)-PHB/C cuboid-bar and PLA/(R,S)-PHB/F film samples released to the degradation medium after 14 and 56 days of incubation at 70 °C.

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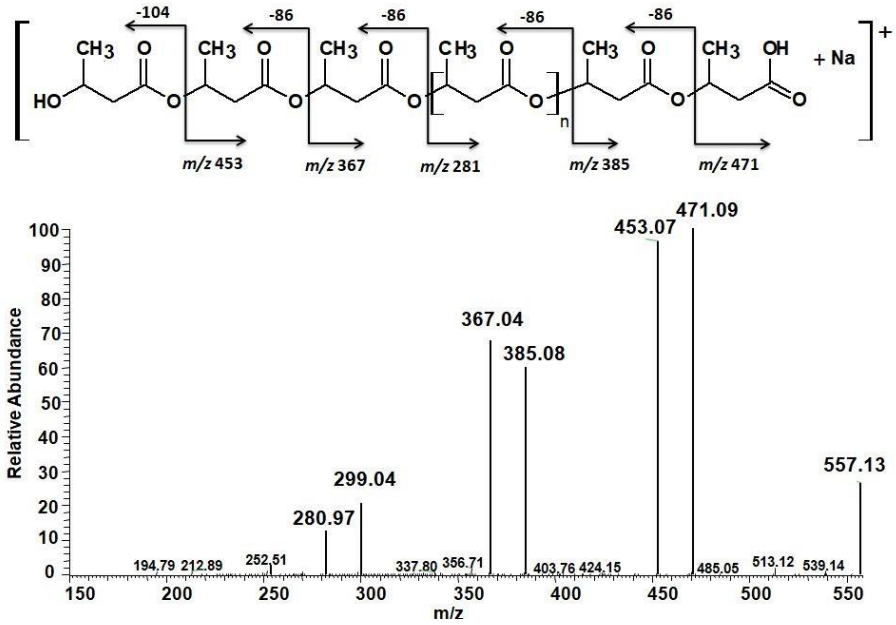

548

549 *Figure 9. ESI-MS/MS product ion spectrum obtained for the sodium adduct of OHB with*

550 *hydroxyl and carboxyl end groups at m/z 557 (Figure 8)*

551

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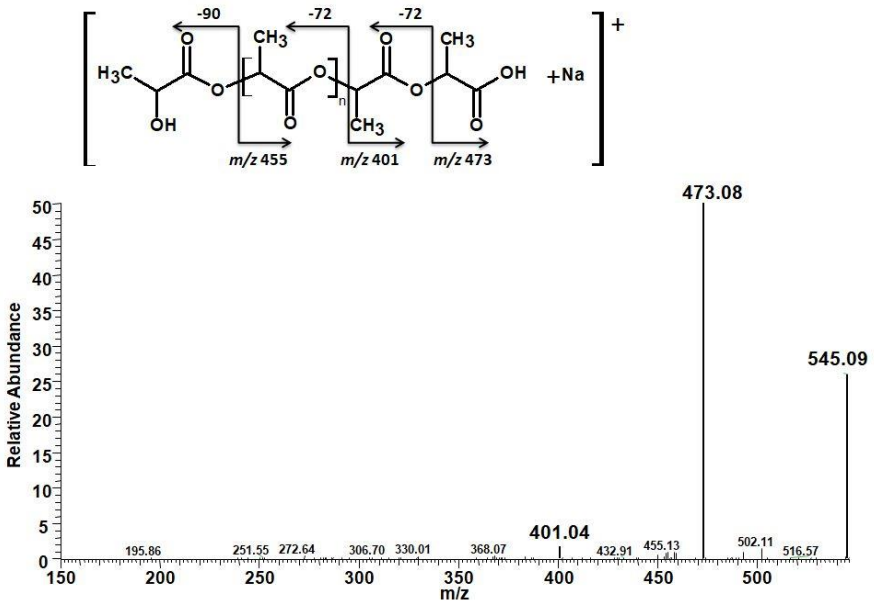

553

554 *Figure 10. ESI-MS/MS product ion spectrum obtained for the sodium adduct of lactic acid*

555 *oligomers with hydroxyl and carboxyl end groups at m/z 545 (Figure 8)*

556

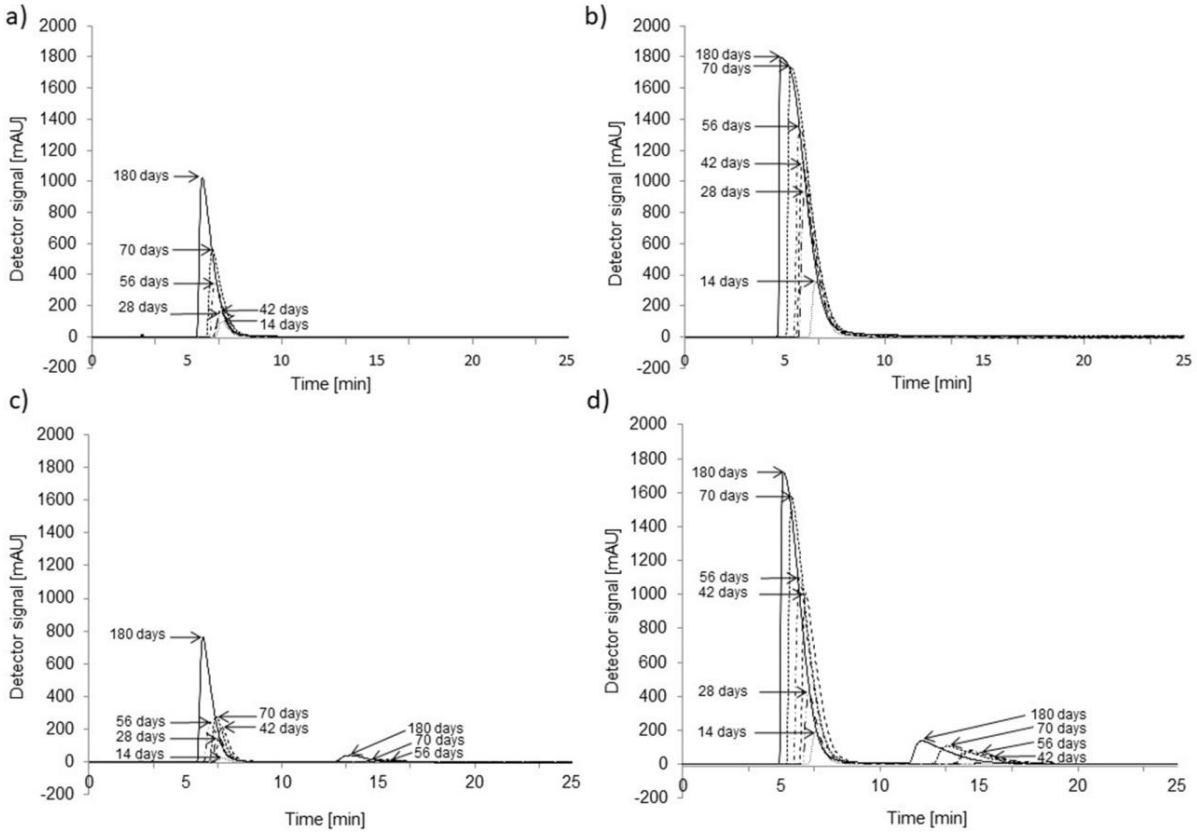

Figure 11. The HPLC chromatograms of the water-soluble degradation products released to the water after 14, 28, 42, 56, 70 and 180 days of incubation for a) PLA/F film sample, b) PLA/C cuboid-bar sample, c) PLA/(R,S)-PHB/F film sample, d) PLA/(R,S)-PHB/C cuboid-bar sample

564

565 *Table 1. The changes of chemical composition of PLA/(R,S)-PHB samples (solid part) after*

566 *specific period of degradation (from ^{1}H NMR analysis)*

Incubation time [days]	**Sample composition [%]**	
	PLA/(*R*,*S*)-PHB/F	**PLA/(*R*,*S*)-PHB/C**
0	85/15	85/15
14	81.1/18.9	77/23
28	82.2/17.8	82.4/17.6
42	86.4/13.6	83.3/16.7

567
568

569

570 *Table 2. Number-average molar mass and dispersity for PLA samples studied during*

571 *incubation in water at 70 ºC*

Sample		Incubation time [days]	
		14	**56**
PLA/F	Number-average molar mass [g/mol]	2100	1100
	Dispersity index	2.4	1.5
PLA/C	Number-average molar mass [g/mol]	1800	1100
	Dispersity index	2.4	1.5

572

573

574 *Table 3. The normalized concentration of lactic acid in degradation medium and total mass*
575 *loss of the sample during degradation time. The normalized concentrations were calculated*
576 *for 1 g of degraded sample*

	Sample	Time of degradation [days]					
		14	**28**	**14**	**56**	**14**	**180**
PLA/F	Concentration [molL^{-1}]	0.0794	0.1843	0.2873	0.3736	0.5272	0.8755
	Total mass loss of sample [%]	29	34	48	57	81	100
PLA/C	Concentration [molL^{-1}]	0.0767	0.2106	0.2478	0.4490	0.5135	0.7937
	Total mass loss of sample [%]	18	34	51	59	89	100

577
578

Table 4. The normalized concentration of lactic and 3-hydroxybutyric acids in degradation medium and total mass loss of the sample during degradation time. The normalized concentrations were calculated for 1 g of degraded sample

Sample		Time of degradation [days]					
		14	**28**	**42**	**56**	**70**	**180**
PLA/(*R,S*)-PHB/F	Concentration LA [molL^{-1}]	0.0070	0.0998	0.1658	0.1794	0.2282	0.6372
	Concentration 3-HBA [molL^{-1}]	x	x	x	0.0415	0.0496	0.1608
	Total mass loss of sample [%]	6	28	48	58	78	100
PLA/(*R,S*)-PHB/C	Concentration LA [molL^{-1}]	0.0286	0.0885	0.2333	0.2903	0.3956	0.8820
	Concentration 3-HBA [molL^{-1}]	x	0.0114	0.0781	0.1091	0.1096	0.2602
	Total mass loss of sample [%]	37	50	57	63	87	100